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NUTRITION REVIEWS

March 1955

Early Experiences with Fish Oils—A Retrospect
by Charles E. Bills 65

Clinical Nutrition
Kwashiorkor 67
Influence of Protein Source on Caloric Requirement 71
Variability of Serum Cholesterol and Lipoproteins in Man 72
Vitamin Deficiencies in Patients with Diabetic Retinopathy 74
Oral Health and Nutriture in Tristan da Cunha 76
Sodium Chloride Intake and Occurrence of Hypertension 79

Nutrition Survey
Dietary Habits of Elderly People 81

Experimental Nutrition
Endocrine Regulation of Blood and Plasma Amino Acids 83
Weight Reduction and Tumors in Mice 86
Metabolism of Microorganisms 87
Body Composition in Animals 89
Synthetic Diets for Rabbits, Guinea Pigs and Rats 92
A Factor in Oil Meals Affecting Animal Fat Composition 94

Notes
The Determination of Serum Albumin Concentration 95
Use of Radiation in Food Processing 95
Nutrition and Highway Safety 95
Fetal and Maternal Riboflavin 96
Recent Nutrition Books 96

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NUTRITION REVIEWS

VOL. 13 MARCH 1955 No. 3

EARLY EXPERIENCES WITH FISH OILS—A RETROSPECT

I was fortunate in having my last year of graduate work under Professor E. V. McCollum. This was shortly after his group discovered vitamin D in cod liver oil and just before Steenbock and Hess associated D with ultraviolet light and the sterols. My thesis dealt with the occurrence and properties of the new vitamin and some improvements on McCollum's line test for measuring it. It included the finding that a fish body oil (menhaden) is, like the liver oils, a source of vitamin D.

One of the visitors at Johns Hopkins in early 1924 was E. Mead Johnson, Jr., the baby food manufacturer, who had it in mind to remove cod liver oil from the folk medicine category and to promote it on its merits. We met in the rattery, and, after a few days of deliberation, I agreed to organize a research laboratory for his company at which fish oils should receive further study. I sketched some floor plans, and the architect and builder went to work.

Johnson and I spent six weeks of the summer in Newfoundland, touring the fisheries and learning what we could about the habits of codfish and the production of oil. There we met Jack Drummond, who had come over from London on a similar mission. Drummond was interested to hear more about McCollum's new vitamin, and gave us in exchange much information about the fishing industry. He had recently traced the vitamin A of fish livers to a possible origin in phytoplankton, and he surmised that the vitamin D had a similar lineage, *i.e.*, plankton → little fish → big fish. In view of this I gave particular attention to the collecting of capelin and squid, principal foods of the cod in Newfoundland waters. We made oils from these and a few other species that were fat enough to yield oil by simple rendering. The samples were to be our first study material at the new laboratory.

Delays in construction enabled me to have some additional months in Baltimore, until the end of 1924. This struck me as an opportunity to isolate a specimen of vitamin D from the ample supplies of cod liver oil now available. I learned that Lawson Wilkins, a young pediatrician with whom I shared laboratory space, was bent on the same accomplishment, and, as we were both in a hurry, we joined forces. We got as far as attempting to fractionate the unsaponifiables in a newly improved vacuum still put at our disposal by Colonel E. B. Vedder. At this point the vitamin vanished. Years later, when Brockmann did isolate vitamin D from tuna liver oil, I could better appreciate his achievement. His starting material was hundreds of times more potent than ours, and his principal technic, chromatographic adsorption, was in our day only a botanist's lecture aid.

Professor McCollum contributed to the Mead Johnson laboratory a breeding stock of rats, descendants of those with which he discovered vitamins A and D. The genealogy of this distinguished line deserves mention. Originally assembled for genetic studies, the strain was a mixture of albinos, Irish blacks, and a wild buff sport from the Liverpool docks. Under McCollum at Wisconsin, they were probably the first rats ever to be used in nutritional research. I in turn supplied a branch of the family to Windaus and Holtz at Göttingen and to laboratories elsewhere.

The Evansville laboratory was ready on January 1, 1925. My first assistant was F. G. McDonald, recently graduated from Purdue. During the previous fall he had built up the rat colony to useful size, and we now began twenty-five years of work together. From the

start it was evident that our efforts would be divided between fish oils and irradiation products, not to mention other fields of nutrition with which the company was concerned.

During 1925 and 1926 we examined a number of oils and fats besides the specimens collected in Newfoundland. We were chiefly interested in vitamin D, and at first paid only incidental attention to the better known vitamin A. Some of this work was planned to broaden our knowledge of the occurrence of vitamin D. Some was directed at the problem of origin. Some was primarily quality control on cod liver oil. The control work required an inordinate number of rats, but it gave us valuable experience and data from which we built a quantitative procedure out of what formerly had been a pass-or-fail test.

Our first published survey included assays on the oils of 17 marine species. It fairly well established the concept that fish oils are the natural source par excellence of vitamin D. It covered three new body oils (herring, salmon and sardine) besides the original menhaden oil, each of which was comparable to cod liver oil as a source of vitamin D. We never could make these body oils palatable enough for human use, but the poultry feed people soon developed a great market for them, especially for sardine oil. This was fortunate, for it released more cod liver oil to human consumption at a time when it was almost the only source of medicinal vitamin D.

The survey included all the marine oils then produced in quantity, but it gave no encouragement to a search for others. Three of the oils were equal to cod liver oil, but the only one that was superior was Hess' pufferfish, a curiosity commercially unavailable. Consequently we did little with fish oils from 1927 to 1932, switching to more promising fields, especially the development of synthetic vitamin D. During this period, however, we collaborated with Hess in a study of individual cod livers which brought out the surprising fact that the potency of the oil varies inversely with the fatness of the liver. This was the first intimation that those species of fish which regularly have lean livers may nevertheless be excellent vitamin sources.

In 1931 the Health Organization of the League of Nations held a conference in London to establish international standards for the vitamins. My assignment was to prepare the definition of the vitamin D unit. At this time I again met Drummond and learned that halibut liver oil exhibits an exceptional vitamin A potency, although the yield of oil is small in comparison with cod. It now seemed in order to take another look at the lean liver species, and so the program was revived, this time with a strictly Edisonian approach designed to include every fish of major commercial importance.

Our laboratory staff had grown considerably, and was now enlarged for this undertaking. J. C. Wallenmeyer was the procurement man who visited the fisheries on both coasts and arranged for agents in Japan (our principal source), the Philippines, and southern hemisphere. He devised an extraction process in which liver was liquefied by alkalinizing to a critical pH and the oil recovered centrifugally. Thus it became possible to recover vitamins from the leanest livers, and by scrubbing the aqueous phase with cod liver oil a high degree of efficiency was realized. Wallenmeyer and I built a vitamin A spectrophotometer which saved countless hours of animal work.

The program got under way early in 1932, and by 1934 we had data on more than 100 species. European workers had noted an interesting correlation between zoologic order and vitamin storage in liver, namely, that the cartilaginous fishes (sharks, etc.) store little vitamin D. We confirmed this, and found several new relationships. The flatfishes (halibuts, soles) were rich in vitamin A; good to poor in D. The rockfishes were mostly superior sources of both vita-

mins. The codfishes, in comparison with most other groups, were only mediocre sources of vitamin D. But above all were the percomorphs (tunas, basses, etc.), most of which carried extraordinary concentrations of vitamins D and A, even hundreds of times more than the cod.

It was obvious that by blending the liver oils of certain percomorph fishes we would have a product of considerable appeal to our physician-customers. But what to call such a product, briefly and definitively without resort to a trade name? Sensing the penchant of doctors for classical terminology, I wanted something reminiscent of Oleum Morrhuae. The local Latin teacher, Miss Georgia Flood, came up with Oleum Percomorphum, a name which probably contributed much to the commercial success of the product.

Only those in the pharmaceutical business can appreciate the problems which beset a new product. To illustrate: A physician wrote to inquire if "O.P.," used as a food adjunct, is kosher, *i.e.*, if all the species involved are fishes with scales. He knew that one of the component species is endowed with scales when young but loses them at maturity—what, then, is the status? The answer, according to the highest rabbinical authority, was that once a fish is kosher, it so remains.

The fishermen of the world soon had a bonanza. Livers formerly regarded as offal were in such demand that in some species they sold for more than the rest of the fish. The highest price that I recall was $8 a pound for frozen ishinagi liver, the tonnage of which was rather limited. Each species had its price, which we revised from time to time on the basis of assays and oil yield. Our problem now was to dissuade the fishermen from palming off worthless livers for the desired ones. Happily, we soon discovered that a good way to recognize a fish is by its liver. The shape, color, markings, relative size of lobes, and pattern of venules are highly specific, and by these criteria one can differentiate species that are confusingly similar in external appearance. A set of preserved liver standards was used to train our inspectors, who could then sort quantities of freshly thawed material at a glance. This method of distinguishing species is still in use by some of the fish and game authorities.

The percomorph liver oils richest in vitamin D are those from the various tuna species. In 1934 we assayed one of these by Massengale's rat-chick method, and were thereby able to demonstrate the existence of more than one form of vitamin D in nature. Later, when Brockmann isolated vitamin D from tuna liver oil, it proved to be a mixture of D_2 and D_3.

We never could show how the fish gets its vitamin D. The capelin and squid oils from Newfoundland did not account for the vitamin in the cod. Some catfish that we raised in captivity without a source of vitamin D seemed to accumulate as much as though they had received it, which would point to a synthesis. But some cod that we dosed with ergosterol, and cod livers digested with ergosterol, showed no synthesis. The question is still open.

Synthetic vitamin A has in recent years joined D_2 and D_3 in competition with fish oils, but fishing is an old art likely to continue, and the fish oils which we explored in 1924–1934 will serve in nutrition for a long time.

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KWASHIORKOR

Kwashiorkor is one of the major nutritional disorders existing in the world. This malady occurs in children in the weaning and postweaning ages in many tropical

and subtropical parts of the world. The diets prevalent in these areas are low in protein, especially that of animal origin, and what little protein is in the diet usually comes from one cereal, either corn or rice. Kwashiorkor and related nutritional syndromes probably result from protein malnutrition.

Children with kwashiorkor display retarded growth, loss of appetite and interest, alterations in skin and hair pigmentation, edema, and frequently associated dermatoses. Gastrointestinal disturbances and anemia may also occur. The disorder carries a high mortality rate in the absence of adequate dietary treatment. Fatty infiltration, cellular necrosis, or fibrosis of the liver are frequently found at autopsy (*Nutrition Reviews* **10,** *261, 297 (1952)*).

The subject of kwashiorkor is reviewed and some original contributions and the viewpoints of several active workers in this field from abroad are presented in a symposium concerning nutritional factors and liver disease which was sponsored by the New York Academy of Sciences. A survey of the world situation on kwashiorkor is given by J. F. Brock (*Ann. N. Y. Acad. Sci.* **57,** *696 (1954)*; *Nutrition Reviews* **13,** *1 (1955)*). The epidemiology of this disease in relation to cirrhosis of the liver and to primary carcinoma of the liver is discussed. There is enough correspondence between incidence of these conditions in some areas to suggest an etiologic relationship, but there are also important areas of noncorrespondence. For example, on the African continent the correspondence between the incidence of kwashiorkor and related malnutrition syndromes to cirrhosis and carcinoma is good. However, although kwashiorkor occurs with significant frequency in Central and South America and the West Indies, the incidence of primary carcinoma and of adult cirrhosis is relatively low. Likewise, a difference between tropical and nontropical areas with regard to the incidence of cirrhosis is apparent, but not so great as one would expect from the frequency of occurrence of primary liver carcinoma in the former areas.

Whether the noncorrespondence of the incidence of these diseases of the liver result from real or apparent differences remains to be proved. The differences might possibly be accounted for by discrepancies in diagnostic and pathologic criteria, and by the unavailability of adequate records of the kwashiorkor, cirrhosis and carcinoma rates based on biopsy or autopsy incidence for some of the areas in question. These problems must be solved before it can be positively stated that "American kwashiorkor" and "African kwashiorkor" are the same disease. In addition, further investigation of the areas of noncorrespondence may provide additional information concerning the pathogenesis of different types of liver disease. As Brock suggests, it may be that the cirrhosis and carcinoma that occur as apparent sequelae to kwashiorkor in Africa may result from continuing cirrhotogenic agents such as hepatotoxic alkaloids, or from a deficiency of unknown factors in the diets of Africans to which adolescents and adults in the Americas are not exposed.

In order to evaluate the role of protein and of labile methyl groups in fatty liver and cirrhosis, B. M. Nicol (*Ann. N. Y. Acad. Sci.* **57,** *764 (1954)*) surveyed three West African tribes (Ijaws, Isokos, and Dakarkerris) and a group of wealthy native traders of similar racial background. Data were gathered on the incidence of hepatomegaly, the histologic appearance of the liver lesions as observed on needle biopsy specimens, the prevalence of parasitic infestation, and alcohol consumption. A dietary survey was done simultaneously. Dietary intakes of essential nutrients, of calories, and of protein were estimated, and the essential amino acid composition of the diets was calculated. Choline intake was also calculated. The Isokos had the

highest incidence of hepatomegaly (19 per cent) and were distinguished from the other groups by a low intake of protein. The incidence of parasitic infestation was approximately equal in the three tribal groups. The group of traders had the lowest incidence of hepatomegaly (3 per cent), and the highest alcohol consumption. From the dietary calculations that were done, no evidence was obtained to indicate that lack of essential amino acids or of labile methyl groups was involved in the development of fatty liver and cirrhosis in the population groups studied.

The pathologic sequence of events which is seen in kwashiorkor, and "which results in permanent alterations to the structure of the liver" is described by J. N. P. Davies (*Ann. N. Y. Acad. Sci.* **57,** *714 (1954)*). A selective atrophy of enzyme-secreting cells of the pancreas, small intestine, and salivary and lacrimal glands, organs with a rapid turnover of protein, is the basic lesion. Early in the disease a marked fatty infiltration of the liver occurs. The cause of the atrophy and its relation to the changes in the liver are not precisely known, but there is some analogy to the development of fatty livers in depancreatized dogs maintained on insulin (I. L. Chaikoff, C. Entenman, and M. L. Montgomery, *J. Biol. Chem.* **168,** *177 (1947)*), and which presumably result from a lack of pancreatic enzymes with subsequent disturbances in the absorption of lipotropic agents.

Attempts to produce in experimental animals the pancreatic lesions of kwashiorkor have not been successful. In kwashiorkor fat appears first in the peripheral cells of the liver lobule. Inflammatory cells, especially lymphocytes, are seen in the portal triad and to some extent in the sinusoids. The periphery of the liver lobules and the portal triads show a reticular thickening and a fibrotic process. This "stellate fibrosis" presents a persistent lesion which Davies thinks may continue to progress in the absence of fat from the liver. The amount of fat in the liver may vary independently of the degree of fibrosis. Although the relationship of one to the other is not clearly understood, the majority of Africans over two years of age have livers which demonstrate cellular infiltration and fibrosis. A continuing lesion initiated in childhood, or the continuing effects of dietary factors in adolescence and adulthood may explain the high incidence of liver cirrhosis observed in the adult African population.

Another point of view is taken by J. Gillman and C. Gilbert (*Ann. N. Y. Acad. Sci.* **57,** *737 (1954)*). These workers express the opinion that there is not a direct relationship between fatty liver and fibrosis of the liver in Africans suffering malnutrition. This opinion is based on their findings that the fatty liver is of acute onset, that the fat usually disappears from the liver, that evidence for a persisting fatty change is not found in sampling of livers of all age groups in the general population, and that the incidence of hepatic fibrosis in the general population does not correlate with the frequency of fatty change. These authors prefer to disengage fibrosis from fatty change, and to consider the two changes as results of separate metabolic processes not necessarily related.

Following a detailed description of the clinical findings in kwashiorkor, H. C. Trowell (*Ann. N. Y. Acad. Sci.* **57,** *722 (1954)*) discusses the treatment of this condition. Mortality rate in untreated cases is almost 100 per cent. Secondary infections, extreme loss of appetite, difficulty in digesting food, and intolerance to fat and certain carbohydrates make management difficult. The latter are probably attributable to the pancreatic and gastrointestinal lesions which occur early in the disease. The feeding of skim milk powder with suitable carbohydrate is the treatment of choice. Ripe bananas are well tolerated as they are in celiac disease. Treatment must be initiated slowly,

and gradually intensified. A response occurs in a few days as indicated by increase in alertness and appetite, onset of a diuresis with loss of edema, and healing of the skin lesions. Fat may persist in the liver for several weeks, and complete nutritional rehabilitation of the patient may require many months. The very slow weight gain seen in children recovering from kwashiorkor is in contrast to the relatively rapid gains in body weight seen in children recovering from uncomplicated undernutrition and caloric deficit. The reasons for these differences are not apparent. It is possible that an anatomic or biochemical lesion may persist in children with kwashiorkor which makes for the inefficient utilization of food, and especially of protein. Elucidation of this phenomenon awaits further metabolic study. Specific agents of potential therapeutic value are difficult to assess, but methionine, choline, vitamin B_{12}, folacin and other known vitamins of the B-complex have not been shown convincingly to have exerted a specific therapeutic response.

A number of biochemical alterations have been noted in patients with kwashiorkor (*Nutrition Reviews* **12,** *5 (1954)*). Decreases in serum albumin levels, in blood cholesterol, alkaline phosphatase, urea, and in serum amylase and esterase have been noted. Dietary protein deficiency and pancreatic insufficiency are probably responsible for these changes. The abnormalities revert to normal following adequate treatment of the syndrome with animal protein. Plasma vitamin E and carotenoids are also decreased in kwashiorkor (H. C. Trowell, T. Moore, and I. M. Sharman, *Ann. N. Y. Acad. Sci.* **57,** *734 (1954)*). This is probably related to the impaired absorption of fat and fat-soluble substances which occurs with this disorder. Another approach to the biochemical investigation of kwashiorkor involves the application of micromethods of enzyme and chemical analyses, utilizing Cartesian diver technics and spectrophotometry, to the study of small samples of liver tissue obtained by needle biopsy (J. C. Waterlow and S. J. Patrick, *Ibid.* **57,** *750 (1954)*). Preliminary results of these studies relating to lactic, malic and glutamic dehydrogenases, cytochrome-c reductase, transaminase, succinoxidase and choline esterase are presented. The concentrations of choline esterase and succinoxidase were decreased, but the other enzymes were well maintained in the fatty liver specimens studied. Malic dehydrogenase and transaminase concentrations were usually increased in the malnourished liver as compared to the values in liver specimens obtained from the patients after treatment. The significance of the unusual finding of an increase in enzyme activity in tissue which was presumably protein-depleted awaits further elucidation. In this study striking differences between the enzyme activities of human liver and of rat liver were found. The need for establishing normal values for the chemical composition of the particulate fractions and for the enzyme content of human liver tissue is obvious.

The importance of kwashiorkor and related malnutrition syndromes as disorders which affect a large segment of the world population is unquestioned. Although much has been accomplished toward a better clarification and understanding of these syndromes, the opportunities for continued and fruitful investigation are great. Kwashiorkor responds favorably to the feeding of dried skim milk powder. Whether this syndrome results from some discrepancy between intake of protein and caloric intake or intake of other dietary factors, from a deficiency of amino acids or an amino acid imbalance, or from deficiency of some as yet unidentified factor or factors contained in milk powder and in animal protein will be awaited with interest. Not until the exact

cause of kwashiorkor is established is it likely that it can be prevented in areas in which it occurs where potent economic factors or racial custom limit the variability and availability of proteins, including those of animal origin.

INFLUENCE OF PROTEIN SOURCE ON CALORIC REQUIREMENT

The quantitative amino acid requirements of man have been studied extensively by W. C. Rose and associates (*see Nutrition Reviews* **8,** *118 (1950)*). The nitrogen balance technic was employed. Subjects were given mixtures of crystalline amino acids and the concentration of the amino acid in question was progressively reduced until the subject was in negative nitrogen balance. The quantity of the various essential amino acids required for the maintenance of positive nitrogen balance was determined in this manner. In such experiments it is extremely important that adequate calories be supplied. When faced with an inadequate supply of calories the organism will utilize amino acids as a source of energy and the nitrogen will be excreted in the urine.

Early in the course of their experiments Rose and his co-workers observed that with adequate nitrogen intake in the form of purified amino acids the caloric requirement for the maintenance of nitrogen equilibrium appeared to be higher than was predicted on the basis of known energy requirements of men on natural diets. This raised the question of the influence of nitrogen source on the energy requirements. This problem has been studied by W. C. Rose, M. J. Coon, and G. F. Lambert (*J. Biol. Chem.* **210,** *331 (1954)*). The experimental procedure consisted of determining the energy requirement for the maintenance of nitrogen equilibrium by subjects receiving nitrogen in the form of purified amino acids, hydrolyzed casein, and intact casein. All diets supplied the same quantity of nitrogen which was an amount previously shown to be adequate for man when added to an otherwise complete diet. Two types of amino acid mixtures were employed. One consisted of the essential amino acids plus glycine and urea and the other consisted of all the amino acids in the same proportions as they occur in casein. Both acid-hydrolyzed and enzymatically hydrolyzed casein were used in various experiments. The acid-hydrolyzed casein was supplemented with tryptophan. The caloric intake was varied by changes in the amount of starch, sucrose, and butter fat fed to the subjects.

When whole casein was fed it was found that 45.5 calories per kilogram of body weight resulted in positive nitrogen balance. In contrast, when the same quantity of nitrogen was supplied in the form of the essential amino acids plus glycine and urea and with the same energy intake the subject was in negative nitrogen balance. Increasing the caloric intake to 55 calories per kilogram of body weight resulted in positive nitrogen balance with the essential amino acid mix.

In another series of experiments it was found that when the nitrogen source was whole casein 35 calories per kilogram of body weight permitted positive nitrogen balance. When either enzymatically hydrolyzed or acid-hydrolyzed casein supplied the nitrogen, nitrogen balance was negative with an intake of 35 calories per kilogram but became positive when the caloric intake per kilogram of body weight was increased to 45.5. It thus appeared that the caloric requirement for nitrogen equilibrium was progressively increased when the nitrogen was supplied as whole casein, hydrolyzed casein and crystalline amino acids.

These findings were confirmed in experiments with 2 other subjects and in addition it was found that the amino acid mix con-

taining twenty amino acids gave the same results as did the mix containing only the essential amino acids with glycine and urea.

The biochemical explanation for these observations remains to be elucidated; however, certain practical applications should be pointed out. Both hydrolyzed casein and purified amino acids are used extensively for parenteral protein administration. These experiments indicate that under such conditions the caloric intake should be increased considerably over what is usually considered adequate if nitrogen equilibrium is to be maintained.

VARIABILITY OF SERUM CHOLESTEROL AND LIPOPROTEINS IN MAN

Recent research in the field of atherosclerosis (*Nutrition Reviews* **12,** *325 (1954)*) has directed attention to the levels of circulating serum lipids and lipoproteins as possible factors related to the development of this illness. If further studies confirm this impression, the accurate evaluation of serum lipids and lipoproteins in man would assume considerable clinical importance. Of equal importance would be a careful evaluation of the spontaneous biologic variation of the levels of these entities in the serum and the extent of the technical error of measurement. This latter question has been studied by D. M. Watkin, E. Y. Lawry, G. V. Mann, and M. Halperin in an investigation reported in *J. Clin. Invest.* **33,** *874 (1954)*, in which the extent of variability of both serum cholesterol and certain species of lipoproteins was measured in adults of differing ages.

A total of 68 male subjects ranging in age from 26 to 92 years was used in this investigation. For the most part, these patients were in the wards of a hospital for chronic disease, although some of the subjects were normal ambulatory young adults working in various academic activities. The subjects were free from the following illnesses: diabetes mellitus, hepatic insufficiency, nephrotic syndrome, hypothyroidism or hyperthyroidism, known malignant disease, acute infection within two weeks, severe anemia, cachexia, obesity, and active syphilis or tuberculosis. None had undergone a surgical procedure, hormonal treatment, irradiation therapy, or anticoagulant therapy within the previous sixty days. No effort was made to select or reject patients on the basis of atherosclerosis because of the obvious defects in technics for the estimation of the extent of atheromatous disease.

Duplicate determinations of serum cholesterol and serum lipoproteins were made twice weekly for ten weeks on 33 of the subjects. The other subjects in the study had single determinations of these substances made at similar intervals. The diet consumed by the hospitalized patients during the period of observation averaged 60 g. of protein, 66 g. of fat, and 217 g. of carbohydrate, yielding a total of 1700 calories. This was a house diet fed ad libitum, and measurement of daily variation showed the intake to be constant within about 10 per cent. The blood samples were drawn prior to breakfast at least fourteen hours after the last meal. Total serum cholesterol was measured by the method of L. L. Abell, B. B. Levy, B. B. Brodie, and F. E. Kendall (*J. Biol. Chem.* **195,** *357 (1952)*), and the serum lipoprotein determinations were done according to the technic of J. W. Gofman *et al.* (*Science* **111,** *166 (1950)*). Patterns obtained in the ultracentrifuge were read to obtain areas for the lipoprotein bands S_f12 to 20, S_f21 to 35, and S_f35 to 100. The range of values obtained in the entire group was as follows: for serum cholesterol, 139 to 323 mg. per cent; for S_f12 to 20, 12 to 88 mg. per cent; for S_f21 to 35, 2 to 42 mg. per cent; for S_f35 to 100, 3 to 119 mg. per cent.

The subjects were grouped according to age in the categories 26 to 41 years, 47 to 59 years, 61 to 69 years, 73 to 79 years, and 80 to 92 years. The authors point out that their sampling was not random since some effort was made to include as broad a range of values within each age group as possible. No significant difference in the means of serum cholesterol or serum lipoproteins was detected in any of these age groups, although in larger, more random groups it has been established that both total serum cholesterol (A. Keys *et al.*, *J. Clin. Invest.* **29,** *1347 (1950)*) and serum lipoproteins of the S_f12 to 20 group (H. B. Jones *et al.*, *Am. J. Med.* **11,** *358 (1951)*) increase with age through the sixth decade.

The data obtained in this study were subjected to an analysis of variance in order to determine the total variation, the variation due to technical error of determination, and the biologic variation. The technical error was obtained from the variance between duplicate determinations on coded aliquots of the same samples and this value was used to adjust the total variance in order to obtain residual or "biological variation."

When the degree of variation between duplicates of both total cholesterol and the three groups of serum lipoproteins was plotted against their respective mean levels, regression lines of significant slope were obtained in all cases. These equations indicated that the standard error of duplicates increased about 0.06 mg. per cent for each milligram per cent increment in mean level of all the lipoprotein fractions and 0.08 mg. per cent for each milligram per cent increment in total cholesterol. When similar regression lines were plotted for total variation as a function of mean level of serum cholesterol and the three lipoprotein fractions, significant slopes were found for the three classes of lipoproteins but not for total cholesterol. These data suggest that the total variability as well as the technical error of evaluation increase with increases in the mean level of serum lipoprotein in all categories studied, whereas only the technical error of total serum cholesterol determination increases with increases of mean level. These effects, furthermore, appeared to be consistent in all age groups.

The actual magnitude in the biologic variation ranged between 12 per cent and 25 per cent of the mean for the lipoprotein fractions and from 6 to 10 per cent of the mean for total serum cholesterol. Inspection of the available clinical information obtained during the period of observation revealed no explanation for the origin of this biologic variation. No importance could be attributed to changes in body weight, to changes in environmental temperature, to the presence of intercurrent respiratory infections, or to differences in physical activity. The authors observed evidences that cyclic trends in the serum lipids sometimes occur. They were unable to explain or predict when these would occur, but they point out the hazard that such spontaneous trends offer to serial observations of the effects of treatment.

The data of this study were also employed to estimate the number of samples required to yield an average level of any desired probability within any specified distance of the true mean. Levels of 50 mg. per cent for S_f12 to 20 molecules and 250 mg. per cent for total cholesterol were considered to be the approximate upper limits of "normal." From statistical formulations it was concluded that a single sample of serum analyzed for S_f12 to 20 molecules at the level of 50 mg. per cent would give an answer within 10 mg. per cent of the true mean 67 per cent of the time. Triplicate analyses would decrease this error to ± 5 mg. per cent. In the case of total serum cholesterol, a single determination would give an answer within 15 mg. per cent of the true mean 67 per cent of the time. Duplicates would reduce this error to within 10 mg. per cent. These estimates are in essential agreement with those advanced by

Jones *et al.* (*loc. cit.*) for lipoprotein measurements.

It should be recognized, however, that transposing these data to another laboratory is fraught with uncertainty unless the technical error of measurement has been demonstrated to be the same in the laboratory concerned. Similar calculations should be carried out in each laboratory using observed technical variation before a true estimate of probable error of a single determination is made.

From the data available in this paper, the authors were unable to conclude that a positive correlation existed between total cholesterol level and the levels of any or all of the lipoprotein groups measured. They admit this may be due to a bias in the sampling which was purposely introduced to include a maximum span of levels, or it may be due to the smallness of the sample. One interesting conclusion derived from this study is that the homeostatic mechanism for maintaining cholesterol and serum lipoproteins at a level characteristic for the individual appears to be similar in patients of all age groups.

This study constitutes an interesting contribution to the field of atherosclerosis because it represents an attempt to define more accurately both intrinsic biologic variability of serum lipids and the errors of methods being used in the study of this problem.

VITAMIN DEFICIENCIES IN PATIENTS WITH DIABETIC RETINOPATHY

In 1936, P. Kimmelstiel and C. Wilson (*Am. J. Path.* **12,** *83* (*1936*)) reported an unusual lesion which they had observed in the kidneys of certain autopsied diabetic patients. This lesion was characterized by deposition of dense hyaline (glassy, homogeneous-appearing) material between the capillary loops of the glomeruli. Subsequently, the association of such nodular lesions with the clinical triad in diabetic patients of hypertension, proteinuria and edema was generally recognized and the entire complex came to be known as the Kimmelstiel-Wilson (K-W) syndrome. This complication is not infrequent in patients with long-standing diabetes and accounts for an appreciable number of deaths in diabetic patients in early middle age.

Sixty years earlier than the report of Kimmelstiel and Wilson, S. MacKenzie described capillary microaneurysms (sac-like dilatations) in the retina of a diabetic patient at autopsy (*Royal London Ophth. Hosp. Rep.* **9,** *134* (*1877*)). However, it was not until 1943 that A. J. Ballantyne and A. Loewenstein (*Tr. Ophth. Soc. U. Kingdom* **63,** *95* (*1943*)) rediscovered retinal capillary aneurysms as lesions characteristic of diabetes mellitus, and it is only within the last few years that N. Ashton (*Brit. J. Ophth.* **34,** *38* (*1950*)) and J. S. Friedenwald (*Am. J. Ophth.* **33,** *1187* (*1950*)) demonstrated independently that the renal lesions of Kimmelstiel and Wilson occur exclusively in diabetic subjects with retinopathy. Now that the retinal and renal lesions are recognized as being manifestations of the same vascular disease process, and since the large capillary aneurysm can be readily identified by means of the ophthalmoscope, a means is available for the early diagnosis of this potentially fatal complication of diabetes mellitus.

Of particular interest to nutritionists is recent work implicating the adrenal cortex and members of the B-vitamin family in the pathogenesis of the K-W syndrome (B. Becker *et al.*, *Diabetes* **3,** *175* (*1954*)). Clinical evidence indicates that in some diabetic subjects without retinopathy (and hence without K-W lesions) there is a decrease in adrenocortical function, while in diabetic patients with retinopathy the adrenal cortex may exhibit excessive activ-

ity. For example, diabetic subjects with retinopathy tend to excrete more than normal quantities of free oxysteroids in the urine while diabetic patients without retinopathy excrete small amounts of oxysteroids in the urine. Many patients with diabetes fail to display a normal eosinopenic response to corticotropin, while almost all diabetic subjects with retinopathy have a normal eosinopenic response under similar circumstances (Becker, *Ann. Int. Med.* **37,** *273 (1952)*). It also has been reported that diabetic subjects without retinopathy are more insulin-sensitive than the retinopathic group (C. G. Zubrod, S. L. Eversole, and G. W. Dana, *New Engl. J. Med.* **245,** *518 (1951)*).

There is also histopathologic evidence of differences in adrenal function between the two groups. The K-W group have heavier adrenals and display a higher incidence of cortical adenomas than do the diabetic patients without retinopathy and nephropathy. Moreover, when alloxan-diabetic rabbits are injected with cortisone, the incidence of K-W renal lesions increases from zero to 75 per cent (Becker, *loc. cit.*).

F. C. Charalampous and D. M. Hegsted (*J. Biol. Chem.* **180,** *623 (1949)*) have shown that alloxan-diabetic rats are much more susceptible to a deficiency of pantothenic acid than are nondiabetic rats. It is also known that pantothenate-deficient animals display evidence of adrenal insufficiency in a manner analogous to diabetic patients without retinopathy. Accordingly, Becker and co-workers (*loc. cit.*) administered large amounts of calcium pantothenate to a series of 11 diabetic patients without retinopathy who had failed to demonstrate any eosinopenia following repeated corticotropin administration. In 8 of the 11 patients, pantothenate treatment was rapidly followed by a normal response to corticotropin. From these and related studies it seems clear that the defective adrenocortical function of at least some diabetic subjects without retinopathy can be attributed to a relative deficiency of pantothenate. Apparently such diabetic patients are in a marginal state of deficiency with involvement of the adrenal preceding that of other organs. Parenthetically, Becker *et al.* found that in 2 patients with pernicious anemia and one patient with sprue who were refractory to corticotropin, administration of 1 g. of calcium pantothenate orally was followed promptly by a normal eosinopenic response to corticotropin. Becker and co-workers have suggested that administration of a metabolic antagonist of pantothenic acid such as omega-methyl pantothenic acid might be of therapeutic value in diseases in which the adrenal cortex is overactive, or in diseases which are alleviated by adrenalectomy.

Diabetic patients with and without retinopathy also appear to respond differently to a vitamin B_{12} tolerance test devised by B. Becker, C. A. Lang, and B. F. Chow (*J. Clin. Nutrition* **1,** *417 (1953)*). Diabetic subjects with retinopathy excrete in the urine a much larger percentage of a standard injected dose of vitamin B_{12} than do those without retinopathy (and also nondiabetic individuals). Since cortisone administration results in a markedly increased excretion of administered vitamin B_{12} in the urine (Becker, Lang, and Chow, *loc. cit.*), the response of the retinopathic patients to the vitamin B_{12} tolerance test is consistent with the hypothesis that such patients suffer from adrenocortical overactivity.

Despite their increased excretion of injected vitamin B_{12}, there is an indication that diabetic patients with retinopathy may have a latent deficiency of vitamin B_{12}. Diabetic patients without retinopathy retain more injected vitamin B_{12} than do normal control subjects. This may be interpreted as indicating that diabetes acts as a conditioning factor in producing latent pantothenic acid and vitamin B_{12} deficiencies in its victims.

When supplementary vitamin B_{12} is withheld from the diets of cortisone-treated,

nonalloxanized rabbits, the incidence of renal lesions (usually 30 per cent) approaches 100 per cent. However, massive doses of vitamin B_{12} fail to prevent production of such lesions by cortisone in 30 per cent of cases. Moreover, improvement of clinical diabetic retinopathy does not occur in patients treated with vitamin B_{12} (Becker *et al., loc. cit.*).

Becker and his associates acknowledge that the apparent deficiency of vitamin B_{12} in diabetic subjects does not result in megaloblastic anemia in such patients. At present there does not seem to be any satisfactory way of reconciling this fact with the evidence under consideration suggesting a deficiency of vitamin B_{12} in diabetic patients. Conversely, it may be wondered why patients with untreated pernicious anemia do not exhibit retinal capillary aneurysms more frequently. In reply to this objection, Becker and co-workers point out that a number of pernicious anemia patients have adrenocortical insufficiency as shown by failure to respond to corticotropin with a significant drop in circulating eosinophils. Pantothenate and not vitamin B_{12} seems to improve adrenal function in such patients; hence, it is suggested that patients with pernicious anemia are protected from retinopathy by some degree of adrenal insufficiency.

The concept proposed by Becker and associates linking diabetic retinopathy and nephropathy on the one hand and adrenal overactivity and abnormalities in the metabolism of pantothenate and vitamin B_{12} on the other is complex and, in many details, admittedly tenuous. However, much of the evidence presented to support the thesis is persuasive and the concept as a whole should provide a stimulus for further studies which may eventually uncover a means of preventing or arresting this serious complication of diabetes mellitus.

ORAL HEALTH AND NUTRITURE IN TRISTAN DA CUNHA

Increasingly few communities exist in the world in this generation which still adhere to local food customs with little or no modification by reason of importation and adoption of the foods which are typical of western civilization. One area which, prior to World War II, had been relatively little affected by refined foods is the tiny island of Tristan da Cunha in the South Atlantic Ocean. A Norwegian Scientific Expedition of 13 men from different fields of interest spent almost four months on the island in 1937–1938 studying the flora and fauna of the island and surrounding waters, the geologic features of the island and the health and habits of the 188 inhabitants. The results of this expedition have been published in the form of 24 monographs, the latest of which is concerned with the oral health and dietary habits of the inhabitants (R. F. Sognnaes, "Oral Health Survey of Tristan da Cunha," *Det Norske Videnskaps-Akademi, 145 pp., Oslo (1954)*). The oral survey included clinical observations, examination of x-rays and plaster casts of the teeth and jaws, salivary analyses, and tests on the histologic, chemical and physical properties of the teeth. In addition, observations were made on the composition of the drinking water and of the common foodstuffs.

The children were somewhat retarded in their dental development with only half of their teeth erupting within the age ranges considered normal by common standards. Comparable retardation was observed in height, weight and ossification. Tooth decay was of a mild and chronic type, mostly affecting pits and occlusal fissures in the molars. Of the 54 children between 1½ and 12 years of age, 2.8 per cent of their deciduous teeth were carious while none

of their permanent teeth had been affected. In the entire population between 6 and 80 years of age, only 7.9 per cent of the permanent teeth were carious. These figures are almost unbelievably low in comparison with the dental caries experience in communities in Northern Europe or in the Americas. The major caries increment was not observed during puberty as is the case in most communities but instead was in older age groups. Another interesting observation was that 50 per cent more carious teeth were found in the young mothers than in young men in the comparable 20 to 30 year age group. Above the child-bearing age there was continually a higher caries incidence among the women than among the men. This higher incidence among the females appeared to have a strong positive correlation with the number of previous pregnancies. This is an observation that is not duplicated in America where the old adage of "a tooth for every child" has been to a large extent disproven.

Binocular and histologic studies of the teeth indicated that there was a rather high incidence of developmental defects. Few of the deciduous teeth and none of the permanent teeth could be characterized as having perfectly formed enamel and dentin. Physical tests indicated that the teeth of the Tristanites had a lower microhardness than comparable teeth of American subjects. Density of the permanent teeth was comparable to values in the literature. However, the deciduous teeth sampled had a lower specific gravity than teeth from other communities. A large number of the anterior teeth of individuals in Tristan da Cunha were described as mottled in a fashion comparable to that in an area where the water supply contains 2 to 3 parts per million (p.p.m.) of fluoride. This was an important finding in view of the fact that the water supply contained around 0.25 p.p.m. of fluoride.

Chemical studies of the teeth provided some interesting data. Phosphorus values appeared to be higher for both enamel and dentin of the deciduous and permanent teeth than values in the literature. Likewise the fluoride levels in the enamel and dentin were appreciably higher than is customary for teeth from subjects consuming a low-fluoride water supply during tooth development.

Periodontal disease was relatively infrequent in this population. Due to lack of oral hygiene and the chronic irritation which was caused by food debris and calculus formation, about half of the population had a mild gingivitis. However, x-ray examination revealed a healthy structure in the alveolar bone except in 10 subjects, where there was a breakdown of the alveolar bone with mobility of the teeth, pocket formation and loss of one or more teeth. Six of these 10 individuals were over 50 years of age and all were over 30 years of age.

The diet of these individuals was extremely restricted in variety and in total amount. The food consumed by three representative families of 14 individuals over a three hundred and forty-one-day period in 1938 was recorded and average analyses were given. Amazingly low caloric intakes were reported with an average of 1227 calories per individual per day. The individuals in one family reported a caloric intake of only 775 calories per day during this period. The major components of the diet were potatoes and fish with an average of 728 and 248 calories per day, respectively. Less than 60 calories per day were consumed from each of the following foodstuffs: crayfish, meat, birds, eggs and milk. The only importation of food was by the haphazard arrival of vessels at the island. The amount of food available through this route was limited both by the infrequency and unpredictability of these calls, and also by the lack of currency or items for barter among the Tristanites. In 1937 this occurred six times and possibly contributed 15 pounds of flour or other cereal products and somewhere between 5 and 10 pounds of sugar per

individual over the entire year. Protein, ascorbic acid, niacin and phosphorus intakes were normal, but in practically every other category the intake was far below the optimum. Vitamin A and calcium were particularly low by commonly accepted standards although it is obvious that no gross evidence of the deficiency of these nutrients was recorded. Insofar as vitamin A was concerned dark adaptation tests did not give any evidence of a clinically definable vitamin A deficiency. Since the weather on the island is wet and cool, little exposure to sunshine was likely. Hence, the vitamin D intake of the islanders must be largely supplied by the high fish consumption even though they discard the fish livers.

The mottling of the anterior teeth and high fluoride content of the teeth are cause for contemplation about the source of the fluorides in view of the low-fluoride drinking water supply. The only logical conclusion seems to be that the source of fluoride in the diet was a result of the high fish consumption. If so, this would seem to be the only record of a sufficiently high food-borne fluoride intake to cause mottled enamel and an elevated fluoride level in the enamel and dentin.

The dental caries experience among the islanders is sufficiently low that it should be commented upon. It seems likely that this is due in part to the fluoride intake. Without doubt another contributing factor is the low availability of sugar, flour and other refined sources of carbohydrate among these individuals. The importance of these factors is all the more striking in view of the poor histologic structure of the enamel and dentin which probably are a result of inadequate nutriture during the periods of tooth development.

The higher incidence of tooth decay among women who have had children and the suggestion of a positive correlation between the incidence of tooth decay and the number of children are facts which are of potential scientific interest. There is no evidence in this country that women who have had children have any higher dental caries experience than women of the same age group who have had no children. In one experiment with rats, females maintained on a mineral-adequate diet through a five-month period had the same incidence of dental caries irrespective of whether they had borne 1 or 2 litters or were unmated (J. H. Shaw, *J. Dent. Res.* **29**, *802 (1950)*). These observations are in keeping with the common belief that the rate of turnover is so low in the components of enamel and dentin that the strains imposed during pregnancy and lactation are insufficient to alter the composition or the caries-susceptibility of the teeth. However, on the basis of the evidence in this monograph, there is a suggestion that in this isolated group of women, the reverse may be true under the metabolic circumstances involved. This raises the question as to whether the bodily economy can be aided by mobilization of some tooth components during the severe stress of pregnancy and lactation on a borderline or deficiency diet. Possibly this can be evaluated in laboratory experiments where the subjects are maintained during pregnancy and lactation on deficient diets rather than on the adequate diet of the above experiment.

The occurrence of *Lactobacillus acidophilus* was explored in all the islanders. Only 39 individuals, 22 per cent of the population, had positive acidophilus counts. Three of these people had no evidence of caries and had very low counts. Another 3 had one or two small grooves in the enamel which appeared to be developmental faults and not active caries lesions. The remaining 33 showed definite evidence of past caries experience or of active carious lesions. The per cent of Tristanites with positive *Lactobacillus* counts increased with increased age until 76 per cent of the individuals in the 50 to 80 year old group had positive

counts, in contrast to 14 per cent for the 0 to 10 year old group. Of the 136 persons who were free of salivary acidophilus, 44 had one or more signs of caries; 8 had lesions of the acute type and 36 showed evidence of a previous carious process. These data suggest that there was a good agreement between open actively carious lesions and the salivary *Lactobacillus acidophilus* counts and that to a large extent the carious lesions preceded the occurrence of high acidophilus counts.

The survey raises various questions about current concepts of dental caries and metabolic circumstances in the teeth and yet provides none of the needed answers. Unquestionably there is real value in surveys of this type and it is unfortunate that more data of this kind are not being collected in meticulous fashion.

SODIUM CHLORIDE INTAKE AND OCCURRENCE OF HYPERTENSION

The observations of a number of investigators have afforded evidence that limitation of salt in the diet may influence favorably the reduction of blood pressure in patients with essential hypertension (*Nutrition Reviews* **9,** *73 (1951)*; **10,** *5 (1952)*; **11,** *33 (1953)*). Other observations concerned with the production of experimental hypertension in animals would seem to indicate that high levels of intake of sodium chloride are important to the development of hypertension (L. A. Sapirstein, W. L. Brandt, and D. R. Drury, *Proc. Soc. Exp. Biol. Med.* **73,** *82 (1950)*; G. R. Meneely, R. G. Tucker, W. J. Darby, and S. H. Auerbach, *J. Exp. Med.* **98,** *71 (1953)*). A recently published report develops the hypothesis that relatively high levels of dietary intake of sodium chloride may have a significant role in the evolution of essential hypertension in man (L. K. Dahl and R. A. Love, *Arch. Int. Med.* **94,** *525 (1954)*).

These authors consider epidemiologic data which demonstrate that hypertension occurs much less frequently in population groups representing relatively primitive civilizations as compared to those from more complex societies. They do not accept the usual explanation of this difference in terms of the strains of living in more highly developed civilizations. In contrast they consider possible factors of diet and state that in all primitive groups for which data are available there is evidence of relatively little dietary sodium, with intakes averaging 2 g. per day or less. With dietary practices like those in the United States the salt afforded in the diet would ordinarily be much greater than this. Only by the total elimination of salt added to foods could such a low level of intake even be approximated.

Dahl and Love, therefore, sought information concerning the quantities of sodium chloride habitually consumed by patients who had developed essential hypertension. Of 28 patients with the disease who were questioned, only one denied the usual addition of salt to food and each of 5 patients with severe cerebrovascular manifestations stated that they used table salt in relatively large quantities for most, if not all, of their adult lives.

The authors studied a larger group of people reporting for annual physical examinations at the Brookhaven National Laboratory. These persons were members of the scientific staff or of the auxiliary services of the laboratory. Five hundred and forty-seven persons were examined and interviewed by one or the other of the authors. Each person was asked about his dietary practices in the use of salt. Three general categories of use were recognized and each individual was placed in one of these groups depending upon his description of

salt consumption. These groups were: (1) *low intake* (persons who denied ever adding salt to food at the table), (2) *average intake* (individuals who added salt to taste at the table), (3) *high intake* (persons who salted most of their food at the table without prior tasting or consideration of the addition of salt in preparation of food). The authors were aware that this classification did not permit quantitative estimates of sodium chloride intake. However, more detailed appraisal of a few diet patterns indicated that persons in the low salt group were consuming about 2 to 3 g. of sodium per day while those in the high intake group were ingesting four to eight times this amount. It was admitted that there was a wide range of intake in the average group and that the comparison of greatest interest was between the low and high salt intake groups.

At the time of physical examination, note was made of the body build and general nutritional state of each person. Blood pressures were taken by a standard procedure and with the subject sitting upright. Any person demonstrated to have a blood pressure on one or more occasions exceeding 140 mm. mercury, systolic, or 90 mm. diastolic was considered to have hypertension. It should be noted that the number of men far exceeded that of women in the group studied, there being 486 men and only 61 women. The age of the subjects was in the range from 18 to 65 years, but the average age was essentially the same in each of the various groups representing different patterns of salt consumption.

The 547 persons studied were found to be divided in terms of habitual use of salt so that 65 persons or 12 per cent were in the low intake group, 243 individuals or approximately 44 per cent in the average intake group and 239 persons or about 44 per cent in the high intake group. If these groups may be considered to be at all representative of the population at large, the observations would seem to indicate that few persons do not use salt as a flavoring agent at the table and that of those who do, there are as many who add salt indiscriminately to food as who salt their food to taste.

There were 41 persons who were found to have elevations of blood pressure sufficient to meet the criteria selected as evidence of hypertension. None was in the low salt intake group. In the average intake group 17 persons or 7 per cent were hypertensive while 24 cases occurred in the high intake group, a prevalence of 10 per cent. Twenty of the 41 individuals with hypertension were noted to be overweight or fat. This proportion was much greater than in the group as a whole.

The significance of the relationships between sodium chloride intake and hypertension in the three groups was tested statistically using the chi square technic. It was demonstrated that the distributions observed might have occurred by chance alone with a probability of less than 3 per cent. In an addendum to the paper the authors record that an additional 350 persons have been observed in subsequent studies and that these are distributed in much the same fashion as in the initial group of 547. Furthermore, the chi square value for the larger group of 897 individuals indicates a probability of chance occurrence of less than 2 per cent in the relationship between salt intake and hypertension.

The authors state clearly that the degree of correlation determined does not establish a cause and effect relationship between sodium chloride in the diet and the development of hypertension. However, the findings are suggestive of an important relationship. The concept is stated that an intake of sodium in excess of an undefined level is a necessary but not totally sufficient factor in the development of essential hypertension. Other factors such as obesity and hereditary predisposition are of unquestioned importance in the evolution of the disease, also.

In clinical experience essential hyperten-

sion occurs somewhat more commonly in women than in men. The observations of Dahl and Love which are cited above pertain more particularly to hypertension in men, since only 11 per cent of the persons studied were women. It should be pointed out, also, that of the women included there was a greater proportion in the low salt intake group than was true of the men. The observations are to be extended in terms of more precise estimates of dietary sodium. It is to be hoped that the correlations under study will be extended to include larger groups of women.

DIETARY HABITS OF ELDERLY PEOPLE

It has frequently been assumed that the dietary patterns of older people, especially those living alone, are molded primarily by such factors as economics and the condition of their teeth (*Nutrition Reviews* **11,** *271 (1953)*; W. H. Sebrell and J. M. Hundley, *p. 179 in* E. J. Stieglitz, "Geriatric Medicine," *J. B. Lippincott Co., Philadelphia (1954)*). A survey of 100 people sixty-five years of age or older (24 men, 76 women) shows that these two factors are not necessarily the most important. M. Jordan, M. Kepes, R. B. Hayes, and W. Hammond (*Geriatrics* **9,** *230 (1954)*) collected food intake data for the above group of people in Westchester County, New York. The survey was made to secure an indication of the customary food habits of older people who were "not restricted in their choice of foods." Sixty-seven per cent of the people were moderately active, with the remainder sedentary.

The results showed that more than half of the individuals did not consume enough yellow and leafy green vegetables to provide the vitamin A intake recommended by the Food and Nutrition Board. Citrus fruits and other foods high in ascorbic acid were inadequate, according to the above standards, in 40 per cent of the diets. Forty-three per cent of the cases showed an intake of less than 1 pint of milk or its equivalent in cheese per day. The consumption of starches and sweets was considerably less than what one is frequently led to believe is the situation. Doughnuts, sweet rolls and coffee cake were eaten by only 33 per cent of the people. Desserts such as cake and pie were included once a day in 32 per cent of the cases, while 37 per cent of the cases reported none. Two thirds of the cases reported negligible amounts of candy and sugar in their diets, with 58 per cent reporting no jams or jellies.

Coffee or tea was used by 89 per cent of the subjects, with about 70 per cent of them consuming from 1 to 3 cups per day. Alcoholic beverages were used by only 34 per cent. Salt restriction was practiced by 31 per cent.

Appetites were reported as good by 67 per cent and fair by 25 per cent. Practically all of the subjects ate three meals a day, with more than half of them reporting no snacks in between meals.

Medical and social factors were listed more frequently than economic reasons for changes in eating habits with advancing years. Medical reasons accounted for changes in eating habits in 41 per cent of the cases, social in 30 per cent, economic in 20 per cent, teeth in 4 per cent, inaccessibility of food stores in 2 per cent, and other factors, mainly lack of cooking facilities, in 9 per cent. Vitamin supplements were consumed by 44 per cent, with 29 per cent of this group doing so on doctors' recommendations. Laxatives were taken routinely by 55 per cent, varying from three times a month to once or more daily.

An extensive survey of the dietary habits of elderly people was made in Sheffield,

England, by E. R. Bransby and B. Osborne (*Brit. J. Nutrition* **7,** *160 (1953)*). The survey included 303 individuals, whose ages were 60 or over for the women and 65 or over for the men. These people were either living alone or living with another elderly person. Records of all food consumed during a one-week period were kept by each subject. A balance and standard tablespoons and teaspoons were provided as a means of standardizing the records. A field worker visited each subject three or four times during the week to insure that the records were being kept properly. The survey was made from January 1950 to March 1951.

Thirteen per cent of the subjects were still employed, but 46 per cent of the group had no other income than the old age pension. Although the absolute amount spent for food increased only slightly with increasing income, the percentage of the weekly income spent on food ranged from 50 per cent for the lowest group to 30 per cent for the highest group. The food records showed that the men ate more bread, cereal, potatoes, root vegetables, cheese, meat, soups, gravies, sugar and preserves than did the women. The women ate more fruit. The apparent discrepancy is due to the fact that the men consumed considerably more calories than did the women. In general, those individuals who lived alone ate less of the foods that required some preparation than did married couples.

From a medical standpoint, only 77 of the 303 people were classified as fit. Men who were so rated ate more breakfast cereal, fruit, green vegetables and puddings than did the rest of the men. With a deterioration of health there was a reduction in the consumption of meat and bacon. As the health of the women worsened, they ate less cakes and biscuits, fish, green vegetables and preserves. For both sexes the amount of soup increased in the diet of the less fit; the amount of milk consumed was practically the same regardless of the state of health.

The individuals with the higher incomes consumed more milk, cakes, biscuits, root and other vegetables, sweets and fruits than did those with the lower incomes. In this particular group, the amount of different foods consumed was dependent to a certain extent upon the income and not the age of the individual. The one exception to this was the consumption of meat, bacon and sausage. All income groups ate the same amounts of these foods. Since the records were made during a period when meat was rationed, this fact and not economics may account for the above similarity.

The dietary intakes were converted into nutrients according to accepted tables of food composition. On the basis of these calculations 12 out of 125 men consumed less than 1500 calories per day. Fifty out of 178 women did likewise. For both men and women the average caloric intake decreased with age. For men the caloric intakes for the different age groups ranged from 2126 to 2006. For women these average caloric intakes ranged from 1811 to 1588.

The average daily protein intake for men was 71 g.; for women, 57 g. The average calcium intake for men was 0.9 g.; for women, 0.8. For men the iron intake was 14 mg.; for women, 10. For men the vitamin A intake was 2340 I.U.; for women, 2115 I.U. For men the thiamine intake was 1.1 mg.; for women, it was 0.9 mg. Riboflavin intake for men was 1.1 mg.; for women, 1.0 mg. The niacin intake was 9 mg. for men; 7 mg. for women. The ascorbic acid intake for both men and women was 21 mg. Except for ascorbic acid, the average intake for nutrients appears to be close to the Recommended Dietary Allowances (*National Research Council Publication No. 302 (1953)*; *Nutrition Reviews* **12,** *240 (1954)*). However, it should be pointed out that for all nutrients there were many individuals who received low intakes. Unfortunately, no correlation was made between the poor dietary habits and the physical condition

of the individuals. It is hoped that the extensive data collected in this survey may eventually be worked up to provide such correlations.

A brief survey was made by these same workers (Bransby and Osborne, *loc. cit.*) of the dietary intakes of 8 men (age 74 to 82) and 8 women (age 69 to 86) who were living in residential homes. These individuals consumed from 400 to 500 calories a day more than the individuals in the preceding group. It is suggested that part of this may be due to the fact that the individuals in the residential homes did not have to prepare their meals.

These two studies of the dietary intakes of elderly people contribute to the information which is urgently needed in order to provide adequately for a segment of the population which is constantly increasing in numbers. These studies complement the earlier survey made among older people in London by M. Pyke, R. Harrison, S. Holmes, and K. Chamberlain (*Lancet* **II,** *461 (1947)*; *Nutrition Reviews* **9,** *304 (1951)*).

ENDOCRINE REGULATION OF BLOOD AND PLASMA AMINO ACIDS

Like many other plasma constituents, the plasma amino acids (other than glutamic acid) vary within narrow limits among healthy individuals. Among the factors which may be concerned with maintaining the plasma amino acids at relatively constant levels are: dietary intake, intestinal absorption, deamination, transamination, and decarboxylation, excretion and reabsorption by the kidney, and transcapillary and transcellular exchange. Since the plasma amino acids are in active metabolic exchange with tissue amino acids, tissue and plasma proteins, and body carbohydrate and fat, it is to be anticipated that changes in the relative activity of various hormones may affect the levels of the plasma and blood amino acids. A number of observations attesting to this have been reported (*Nutrition Reviews* **10,** *99 (1952)*).

J. M. Luck, A. C. Griffin, G. Boer, and M. Wilson (*J. Biol. Chem.* **206,** *767 (1954)*) have studied the effect of single injections of various hormones on blood amino acids in normal white rats, 250 to 350 g. in weight, and in hypophysectomized rats, 100 to 160 g. in weight. The hypophysectomized rats were purchased and placed on experiment two weeks after arrival. All rats were fed a commercial chow diet ad libitum, but were fasted overnight before testing. The hormones were injected subcutaneously, except growth hormone, which was given intraperitoneally.

The amino nitrogen concentration in the blood was measured colorimetrically. The average concentration in normal male rats was somewhat higher than in normal females and in hypophysectomized males and females. There was little difference in blood amino nitrogen between the normal and hypophysectomized females. C. H. Li, I. Geschwind, and H. M. Evans (*J. Biol. Chem.* **177,** *91 (1949)*) have also noted a lowering of plasma amino nitrogen concentration in hypophysectomized male rats.

Luck and his associates confirmed the observation of several investigators that a transient fall in the blood amino nitrogen concentration follows the injection of epinephrine into normal rats. They made the additional observation that hypophysectomized rats respond to epinephrine in a similar but slower fashion. This is interpreted by them to indicate that this action of epinephrine is not mediated through the pituitary.

Norepinephrine was without effect in normal rats, but induced hypoaminoacidemia in the hypophysectomized rats.

Diethylstilbestrol behaved in a similar fashion. No explanation for these effects is offered by the authors.

Growth hormone induced a hypoaminoacidemia in intact rats. This is in keeping with the observation of Li and his associates in regard to the plasma amino acids, and has been attributed to the anabolic properties of this hormone. Luck and his co-workers also found that growth hormone lowered the blood amino acids in hypophysectomized rats.

In another paper, A. C. Griffin, J. M. Luck, V. Kulakoff, and M. Mills (*J. Biol. Chem.* **209,** *387 (1954)*) observed that single injections of thyrotropin and insulin into normal and hypophysectomized rats led to a hypoaminoacidemia similar to that observed following injection of epinephrine or growth hormone. On the other hand, in adrenalectomized rats maintained on saline, neither insulin nor growth hormone had any significant effect, while thyrotropin and epinephrine, particularly the latter, still induced hypoaminoacidemia. This suggests to the authors that epinephrine and possibly thyrotropin, but not growth hormone or insulin, may be final effectors in inducing hypoaminoacidemia.

Norepinephrine, desoxycorticosterone acetate, cortisone, corticosterone, testosterone, diethylstilbestrol and 11-desoxy-17-hydroxycorticosterone did not affect the blood amino nitrogen concentration in adrenalectomized rats. Previously, Luck and his co-workers had observed no effect when testosterone, cortisone, or desoxycorticosterone was injected into normal or hypophysectomized rats.

Of several commercial adrenocorticotropin preparations injected into normal and hypophysectomized rats, ACTHAR (Armour) alone did not affect the blood amino acid levels. ACTH (National), H. P. ACTHAR gel (Armour) and EAPE (equine anterior pituitary extract) lowered the blood amino nitrogen concentration (A. P. Rinfret, A. C. Griffin, and B. C. Wexler, *Fed. Proc.* **12,** *259 (1953)*). Two possible explanations for the divergent action of the different adrenocorticotropin preparations are offered by the authors: (1) "Pure" adrenocorticotropin does not affect the blood amino acids. On this basis, ACTHAR (Armour) represents a "pure" product, whereas the others represent "impure" preparations. (2) Adrenocorticotropin leads to a lowering of the plasma amino acids—on this basis, ACTHAR (Armour) had lost some activity, while the other preparations had retained their potency.

The authors state that they were thus unable to confirm the report of Li *et al.* (*loc. cit.*) "that adrenocorticotropin increases the concentration of amino acids in the blood of rats" (the quotation is from the article of Luck and his co-workers). However, it should be pointed out that there are several differences between the studies of Luck and his associates and that of Li and his coworkers. For example, the latter group measured the amino nitrogen in the *plasma* by a gasometric ninhydrin technic (P. B. Hamilton and D. D. Van Slyke, *J. Biol. Chem.* **150,** *231 (1943)*) while the former measured the amino nitrogen in *whole blood* and by a less specific colorimetric technic (E. G. Frame, J. A. Russell, and A. E. Wilhelmi, *Ibid.* **149,** *255 (1943)*; Russell, *Ibid.* **156,** *467 (1944)*). In their experiments Luck *et al.* did not observe a significant increase in plasma amino acids of normal or hypophysectomized rats following a *single* injection of adrenocorticotropin hormone. A significant increase was noted in normal rats injected intraperitoneally three times a day for four days and in hypophysectomized rats injected daily for fourteen days beginning on the day of hypophysectomy.

A. L. Sheffner and O. Bergeim (*Arch. Biochem. Biophys.* **49,** *327 (1954)*) have also studied the effect of ACTH on amino acid metabolism. They determined the effect of a single intraperitoneal injection of ACTH upon the concentration of ten individual amino acids in the plasma, muscle, liver and

kidneys of normal and hypophysectomized rats. The hypophysectomized rats were studied fifty-six hours after operation. The normal animals were fed a diet of chow ad libitum while the hypophysectomized animals were fed fresh whole milk and vitamin-enriched white bread. The rats were fasted overnight before the experiment, and killed three to four and one-half hours after injection of the ACTH, since preliminary experiments had shown the largest changes in amino acid levels at this time interval. Arginine, histidine, tryptophan, leucine, isoleucine, valine, lysine, methionine and phenylalanine were analyzed by a microbiologic technic.

Among the normal animals there were transient increases in muscle arginine, lysine, tryptophan, leucine and isoleucine. Lysine alone increased in the liver whereas in the kidney there was a marked and more prolonged increase in the levels of all ten amino acids measured.

Small but significant increases in the *plasma* concentrations of phenylalanine, tryptophan, leucine, isoleucine and valine occurred. These increases did not correspond, relatively speaking, to the concentrations of the individual amino acids in rat skeletal muscle protein. In dogs, on the other hand, increases in the blood levels of leucine, valine and histidine proportionate to their concentrations in skeletal muscle was observed by W. D. Lotspeich (*J. Biol. Chem.* **185,** *221 (1950)*) following a single intraperitoneal injection of ACTH. Lotspeich also observed that following the injection of growth hormone or insulin into dogs there was, in general, a direct correlation between the relative rates of fall of ten individual amino acids (leucine, lysine, isoleucine, valine, threonine, arginine, phenylalanine, histidine, methionine and tryptophan) measured and their concentration in skeletal muscle protein. ACTH counteracted the effect of insulin.

When ACTH was injected into the hypophysectomized rats, Sheffner and Bergeim found no increases in concentrations of the amino acids in the plasma or tissues. They point out that the inability of the hypophysectomized animals to respond to ACTH may have been due to multiple causes, among which they mention (1) the previous loss of labile protein which follows surgery, (2) the effects of potassium retention following hypophysectomy. In regard to the latter, they mention the observation of J. E. Whitney and L. L. Bennett (*Fed. Proc.* **9,** *134 (1950)*) who reported that the catabolic effect of ACTH was partially inhibited by increasing potassium intake. On the other hand, Sheffner and Bergeim point out that there may have been a lag in the response of the hypophysectomized animals and that they did not observe a rise simply because the samples were not taken over a long enough period of time.

When renal function is normal, large increases in the plasma levels of amino acids may not occur, since a rise in their concentration above the renal threshold may result in a large increase in their excretion. Urinary amino acid measurements were not made by Sheffner and Bergeim because the experiments were of such short duration that the collection of sufficient urine was not feasible. They observed that the increase of kidney amino acids following the injection of ACTH into normal rats was close to its maximum when the amino acid levels in the other tissues and the plasma were already normal. This suggests to them that the increase in urinary amino acids which has been reported to follow administration of ACTH (*Nutrition Reviews* **10,** *99 (1952)*; E. Ronzoni, E. Roberts, S. Frankel, and G. B. Ramasarma, *Proc. Soc. Exp. Biol. Med.* **82,** *496 (1953)*) may be associated with changes in the free amino acids of kidney tissue rather than an increase of plasma levels.

In seeking an explanation for the aminoaciduria observed in patients with burns or undergoing major operations, G. L. Nardi (*J. Clin. Invest.* **33,** *847 (1954)*; *Nutrition Reviews* **12,** *328 (1954)*) considered the possi-

bilities that the aminoaciduria is a reflection of an increase in the plasma concentration of the amino acids following injury or a decrease in renal tubular reabsorption of amino acids. He suggests that a disorderly, albeit not elevated, pattern of amino acids may be presented to the kidney, leading to a disturbance of tubular reabsorption. Nardi also mentions the possibility of an increased secretion of an adrenocorticosteroid, probably other than cortisone, which would decrease tubular reabsorption of amino acids.

WEIGHT REDUCTION AND TUMORS IN MICE

Although many arguments have been put forth advocating weight reduction of obese individuals, there is surprisingly little experimental evidence, as contrasted with clinical experience and life insurance mortality statistics, to support the avowed advantages of a weight reduction program.

Work with animals is beginning to supply the needed information. S. H. Waxler (*J. Nat. Cancer Inst.* **14,** *1253* (*1954*)) has presented evidence that obese female mice show a lower incidence of spontaneous mammary cancer after their body weight has been reduced. It has been shown (Waxler, P. Tabar, and L. R. Melcher, *Cancer Research* **13,** *276* (*1953*)) that virgin C_3H female mice made obese by the injection of goldthioglucose (*see Nutrition Reviews* **13,** *15* (*1955*)) showed an earlier appearance and a greater incidence of spontaneous mammary cancer than did control mice of normal weights. In the present study, Waxler used the C_3H strain of mice which ordinarily shows a high incidence of spontaneous mammary tumors. He placed 65 virgin females on a Purina chow ration until they were two months of age. At this time 45 mice were given an injection of goldthioglucose. One month later all of the injected mice were obese. At that time 25 of the obese mice were starved down to a weight equal to that of the nonobese controls. From then on the latter two groups were pair-fed in order to maintain the reduced weights of the previously obese mice. The 20 mice which remained obese throughout the study had free access to the Purina chow.

The control animals that had never been obese began to develop tumors at an average age of 267 ± 6.5 days. The obese mice developed tumors at an average age of 219 ± 4.4 days. The mice that had been obese but then reduced developed tumors at an average age of 365 ± 12 days. As in the earlier study, the obese mice developed tumors sooner than the controls. The outstanding finding, however, was the delay in the appearance of the tumors in the mice that had been obese but then reduced. In spite of this difference in the onset of tumors, the survival time after the tumors once appeared was essentially the same in all three groups.

Other workers showed (A. Tannenbaum, *Cancer Research* **2,** *460* (*1942*); M. Visscher, Z. B. Ball, R. H. Barnes, and I. Sivertsen, *Surgery* **11,** *48* (*1942*)) that restricting the food intake of mice markedly delayed the appearance of both spontaneous and experimentally produced neoplasms. Since Waxler's obese mice that had been reduced regained their body weight when offered diet on an ad libitum basis, he suggested that his obese mice on the restricted food intake were constitutionally comparable to starved animals. On this basis, he suggested a similar explanation for the lower incidence of tumors in his previously obese mice and the starved animals of the earlier investigators.

METABOLISM OF MICROORGANISMS

Microorganisms are playing an increasingly important role in the study of nutrition and metabolism. The study of yeast metabolism by Harden and Young some fifty years ago became a cornerstone of our present extensive knowledge of carbohydrate oxidation in living tissues, both plant and animal. The intestinal flora has been found to contribute significantly to the nutrition of the host. The mold *Neurospora* has contributed immeasurably to the elucidation of the gene's influence on metabolic pathways. A number of B-vitamins have been discovered in part because of a search for essential growth factors for microorganisms. The *Lactobacilli*, among others, lend themselves to semiquantitative analyses by microbiologic methods. The search for metabolic inhibitors (antimetabolites, metabolic poisons, enzyme inhibitors, etc.) as possible therapeutic agents frequently involves the use of microorganisms.

A further study of vitamin antagonists has come from the laboratory of D. E. Hughes (*Biochem. J.* **57**, *485 (1954)*). In this study various halogenated derivatives of niacin (niacin compounds in which one hydrogen atom has been replaced by fluorine, chlorine or bromine) were tested for their effects on the metabolism of *Lactobacillus arabinosus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris*. Three criteria were employed in determining the inhibitory effects of the halogenated niacin derivatives: (1) their effect on the growth rate of the bacterial cultures, (2) their effect on the amount of diphosphopyridinenucleotide (DPN) synthesized (DPN is an oxidative coenzyme in which niacin forms a part of the larger molecule), and (3) their effect on the rate of respiration or glycolysis of the organisms.

Niacin-deficient cells were grown in a medium containing minimum concentrations of the vitamin. Growth or rate of cell division was measured turbidimetrically after the niacin-deficient organisms had been allowed to grow further on a medium containing the niacin antagonist as well as niacin itself in various concentrations. Of the 5 niacin derivatives tested, 5-fluoronicotinic acid was the most effective inhibitor of growth for the several microorganisms tested. This compound has an atom of fluorine replacing one atom of hydrogen at the number 5 position of the pyridine ring of niacin. The other halogenated derivatives were more or less active as inhibitors depending upon the type of organism employed. *E. coli*, for example, was inhibited only by 5-fluoronicotinic acid but not by the others. On the other hand, *L. arabinosus* was inhibited by all 5 niacin derivatives tested. Since it might be supposed that the inhibitory action of 5-fluoronicotinic acid depended upon the blocking of the conversion of niacin into DPN, Hughes set up an experiment to see whether the addition of DPN to the growth medium for *L. arabinosus* would overcome the growth inhibition of 5-fluoronicotinic acid. He found that DPN was no more effective than its precursor niacin itself. If 5-fluoronicotinic acid had been blocking the conversion of niacin into DPN, the addition of preformed DPN should have permitted substantial growth of the test organism, provided it entered the cells.

In contrast to this experiment, studies of the rate of carbon dioxide formation by *L. arabinosus* gave the following results: Niacin itself stimulated carbon dioxide formation. This stimulatory effect was abolished by 5-fluoronicotinic acid. DPN even in the presence of 5-fluoronicotinic acid stimulated carbon dioxide production as much as niacin by itself. These experiments suggest that the function of DPN is not inhibited by the 5-fluoronicotinic acid. Actual determination of the amount of

DPN contained in the cells showed that 5-fluoronicotinic acid was capable of inhibiting the synthesis of DPN from its precursors. The degree of inhibition of DPN synthesis was increased at least ten-fold if the bacterial cells were first incubated with 5-fluoronicotinic acid and then subsequently exposed to niacin. The author suggests that 5-fluoronicotinic acid is metabolized by the bacterial cells in a manner analogous to the natural niacin. The products of such "lethal synthesis" might inhibit metabolism at several points.

The mechanisms by which yeasts synthesize protein are not only of great theoretic interest but in the case of the food yeasts may in time assume considerable practical significance. Since the synthesis of proteins or their constituent amino acids requires carbon skeletons derived from carbohydrates and fats, as well as energy to drive the synthetic reactions, E. W. Yemm and B. F. Folkes (*Biochem. J.* **57,** *495 (1954)*) have investigated the interrelation of cellular respiration and the assimilation of nitrogen from inorganic sources such as ammonium salts. The yeast *Torulopsis utilis* was grown on a medium containing sucrose, ammonium phosphate, ammonium sulfate, as well as the other salts required by this organism. Nitrogen-deficient cultures were then prepared by suspending the yeast in a similar medium from which the ammonium salts had been eliminated. Even in the absence of an external supply of nitrogen, the yeast approximately doubled in weight during a twelve- to sixteen-hour period of incubation. Oxygen uptake was measured by the Warburg technic. Quantitative determinations for ammonia, total nitrogen, glutamic acid, glutamine, asparagine, alanine, glutathione, total carbohydrate and the constituent carbohydrate fractions were carried out on relatively large quantities of yeast suspensions. Suspending the nitrogen-deficient yeast in a new medium containing ammonium salts caused a three- to four-fold increase in the rate of oxygen uptake as compared with that of yeast continued on a nitrogen-free medium. A close relationship was found between the increase in oxygen consumption and the rate of ammonia uptake by the nitrogen-deficient yeast suspended in a medium containing ammonium salts. Certain chemical changes occurred in the yeast during the development of the nitrogen deficiency state. There was a great increase in the total carbohydrate stored in the yeast. The soluble nitrogen fraction diminished while the protein nitrogen increased. The rate of oxygen uptake increased, as did the intensity of the stimulatory effect of ammonium salts.

During nitrogen assimilation by the nitrogen-deficient yeast there was an increase in glutamine, glutamic acid and alanine followed by a later increase in protein. Simultaneously there was a decrease in carbohydrate, particularly in glycogen. The close dependence of nitrogen assimilation and protein synthesis upon the oxidative rate was further supported by experiments with dinitrophenol. The chief metabolic action of dinitrophenol is thought to be a diversion of oxidative energy into channels not leading to useful work such as the synthesis of cell constituents. In these experiments, dinitrophenol was found to diminish the assimilation of nitrogen into the various amino acids and proteins.

Glutamic acid plays a central role in the synthesis of other amino acids in that it is capable of donating its amino group for this purpose. It has been suggested that glutamine plays a significant part in the formation of peptide bonds during protein synthesis. These facts may have a bearing on the observations reported by Yemm and Folkes that glutamine and glutamic acid in yeast increase during the uptake of ammonia nitrogen, particularly since this increase precedes the rise in rate of protein synthesis.

E. J. Conway and P. T. Moore (*Biochem. J.* **57,** *523 (1954)*) have reported on the preparation of a yeast in which sodium has

replaced up to 98 per cent of the intracellular potassium. This sodium yeast grew considerably more slowly than yeast containing its normal complement of potassium. Its oxygen consumption also was markedly reduced, although the rate of fermentation remained unchanged. In morphologic appearance and physical characteristics the sodium yeast differed markedly from yeast containing larger amounts of potassium. Since various metal ions play important roles in enzymatic reaction, this technic of replacing one intracellular ion for another in yeast may be useful in helping to elucidate certain problems in the mechanism of enzyme action.

BODY COMPOSITION IN ANIMALS

There is a variety of reasons for the nutritionist's interest in body composition and in the different approaches to this problem. L. J. Pecora and B. Highman (*J. Nutrition* **51,** *219 (1953)*; *Nutrition Reviews* **12,** *61 (1954)*) examined the organ weights in thiamine-deficient, pair-fed, and normal control rats in order to determine the effect of chronic thiamine deficiency (experimental animals) and chronic undernutrition (pair-fed animals) on the changes in organ size. The experimental animals grew normally at first, reaching the weight of 140 g., then gradually lost weight on a thiamine-free but otherwise adequate diet, reaching a weight decrement of about 40 per cent in the next six to seven weeks with a weight of 85 g. This weight was reached by normal rats (controls) at 33 days of age.

The "organ: body weight" ratios for the heart, brain and several other organs were greater in the thiamine-deficient animals than in the controls of the same age but of normal weight (292 g.). These ratios were increased also in the pair-fed animals (weight of 96 g.) which indicates that these differences are not due to thiamine deficiency per se but to food restriction. Only for the kidneys was the ratio substantially higher in the thiamine-deficient than in the pair-fed animals, suggesting that the effect of thiamine deficiency was superimposed on food restriction.

The absolute losses in organ weights, except for the larger kidney of the thiamine-deficient animals, were similar in the two groups. In the pair-fed controls, simple food restriction had little effect on the weight of brain (reduced to 88 per cent of the organ weight of normal fed controls) and addrenals, larger effect on heart, kidneys and testes (40 to 50 per cent of control values), and a very marked effect on the weight of the liver and spleen which decreased to 22 and 26 per cent of the value of the normal controls.

A large amount of current research in animal nutrition is concerned with the effect of antibiotics on animal growth and carcass composition. T. W. Perry, W. M. Beeson, and B. M. Vosteen (*J. Animal Sci.* **12,** *310 (1953)*) studied in swine the growth effects of an antibiotic (7.5 mg. of aureomycin hydrochloride per pound of total ration) and a surface-active agent (0.26 per cent of alkyl benzene sulfonate). Starting with the same average weight (40 pounds), the control animals after 112 days averaged 187 pounds, the aureomycin group 218 pounds, the surfactant group 198 pounds. The effect of the antibiotic on weight gain was statistically highly significant, the effect of the surfactant approached significance. The effect was associated with a higher amount of food consumed (4.5, 6.2 and 5.2 pounds per day for the respective groups). There was no difference in food efficiency (about 3.6 pounds of food per pound gain). The back fat thickness at the first rib was significantly greater at the same kill weight for carcasses of the two experimental groups. The ether extract represented 47.8 per cent of "edible

carcass" in the controls, 56.0 per cent in the aureomycin-fed, and 53.5 per cent in the group receiving the surfactant. Water and protein content were lowest in the aureomycin group.

P. F. Fenton and M. T. Dowling (*J. Nutrition* **49,** *319* (*1953*)) used chemical determinations of carcass fat as a criterion of obesity in mice, believing that body weight alone is not an adequate criterion. They proposed to determine the increase in carcass fat per unit of fat-free weight in obviously lean animals and to consider as "obese" those animals which deviate from the regression line on the positive side by more than two standard errors of estimate. As the selection of "obviously lean animals" is arbitrary, and is based on the fat content of the carcass, the reasoning appears theoretically questionable. The fat content of the body varies within a large range and there are "degrees of fatness." "Leanness" and "obesity" are simply the extremes, defined by arbitrary limits which may vary according to the purpose of the classification. However, the use of fat-free weight as the reference point is definitely preferable to the gross body weight.

Another point brought out by Fenton and Dowling is the fact that deposition of chemically defined fat is associated with an accretion of protein and other nonlipid materials. This strengthens the observations of A. Keys and J. Brozek (*Physiol. Rev.* **33,** *245* (*1953*)) who estimated that during weight gain in adult men the "obesity tissue" consisted of 14 per cent of extracellular fluid, 62 per cent fat and 24 per cent "cells."

Provision of methods for estimation, *in vivo*, of the composition of individual animals during growth and fattening has been a pressing task in genetics and nutritional science. Determinations of body water, body density and the thickness of subcutaneous fat represent relatively new and useful technics for the study of body composition. These methods were applied to 24 hogs (weight range 29 to 155 kg., age range 90 to 466 days) by H. F. Kraybill, E. R. Goode, R. S. B. Robertson, and H. S. Sloane (*J. Applied Physiol.* **6,** *27* (*1953*)). This was a continuation of earlier work on body composition in cattle (Kraybill, O. G. Hankins, and H. L. Bitter, *Ibid.* **3,** *681* (*1951*); **4,** *575* (*1952*)).

Specific gravity measurements were made on eviscerated hogs. It is noted that the values obtained for carcass and viscera were summated to obtain the specific gravity of the total. However, this "total" is a whole animal less the skin, blood and lungs (*J. Applied Physiol.* **4,** *see p. 576* (*1952*)). Consequently, it can not be equivalent to the specific gravity of an intact animal. The data on total body water and fat estimated from antipyrine dilution refer to the living animal.

The terms "specific gravity" (obtained as the ratio of body weight to the *weight* of water displaced on submersion) and "density" (ratio of body weight to body *volume*) are interchanged uncritically. In deriving the formula for calculating body fat from body density, A. R. Behnke's value of 1.100 is used for "lean body mass." This value was derived by Behnke for *man*, on the basis of certain assumptions about the composition of the human body. Kraybill *et al.* did not utilize the opportunity to obtain empirically determined values.

It should be noted, also, that Behnke's lean body mass refers to fat-free body mass plus a certain amount of "essential" fat. Initially, the latter was considered to amount to some 10 per cent. Later, the essential lipids were reduced to 2 per cent (A. R. Behnke, E. F. Osserman, and W. C. Welham, *Arch. Int. Med.* **91,** *585* (*1953*)). In direct chemical determinations, in which fat is obtained by extraction, the remainder must be referred to as fat-free body mass, not lean body mass. This applies also to those situations in which the fat content is determined from antipyrine dilution, on the

assumption of a constant water content in the *fat-free* body mass.

Kraybill *et al.* (*J. Applied Physiol.* **6,** *27* (*1953*)) did not carry out fat extraction and determined "fat" by a physical separation of fatty tissues. The physically separable adipose tissue is anything but pure fat. T. H. Hopper (*J. Agr. Res.* **68,** *239* (*1944*)), in his comparison of methods for the estimation of the physical and chemical composition of cattle, reported that ether extract accounts for 69.8 per cent of the "fat" in the 9–11 rib cut. In man, studied by R. M. Forbes, A. R. Cooper, and H. H. Mitchell (*J. Biol. Chem.* **203,** *359* (*1953*)), only 72 per cent of adipose tissue was fat, while 23 per cent was water.

The data of K. Bernhard and H. Korrodi (*Helvet. chim. acta* **30,** *1786* (*1947*)) on the lipid and water content of man's mesenteric, omental and subcutaneous adipose tissues indicate a good deal lower figures for lipids and a distressingly large range of variation. Part of this variation is certainly due to the heterogeneity of their patients, but the problem is important and definitely needs a reinvestigation.

Kraybill *et al.* (*loc. cit.*) compare fat content estimated from antipyrine dilution and body density with the separable adipose tissue. The values happen to agree fairly closely, on the average (mean values of 36.7, 40.8 and 38.4 per cent of body weight, respectively). The authors failed to indicate that we deal here with heterogeneous, not directly comparable substances and tissues, and that this agreement must be interpreted carefully.

There was a close negative correlation between the percentage of separable fat in the carcass and total body water in the intact animal ($r = -0.954$), and a close positive correlation between separable body "fat" and the fat estimated from total body water ($r = +0.956$). Similarly, "whole animal" specific gravity correlates positively with the percentage of body weight accounted for by body water, negatively with body fat estimated from antipyrine dilution.

There is a positive and fairly high correlation between the percentage of separable fat in the carcass and the thickness of the subcutaneous fat measured along the back of the hog at four anatomic points ($r = +0.811$). This indirect method of evaluation of fatness is applicable to the live animal. Small incisions are made through the skin and the fat layer, and a metal ruler is inserted to a depth where it is stopped by the well defined muscle layer. Although this method lacks the precision of body water (and body density) measurements, it has the advantage of simplicity and rapidity.

The experience with animals larger than guinea pigs and rats, and nutritionally more similar (as the hog is) to man increases our confidence in the application of the indirect methods to problems of human nutrition. It is principally from animal work that we can expect further sophistications in methodology of the indirect study of body composition, including evaluation of factors (such as mineral content) which are considered at present, for the sake of simplicity, as being "constant" but which show differences between individuals.

It is desirable to maintain a constant frame of reference and clearly differentiate between chemically defined fat and the adipose tissue obtained on physical analysis. The disregard of temperature in defining the density of fat or water, and the confusion between specific gravity and density should be no longer tolerated. While the emphasis, in the past, has been on the "constancy" of the average values of the water content of the fat-free body mass, the need is for a critical examination of the composition of this mass in animals differing in their degree of "fatness." The tissue deposited during fattening is complex, containing water and protein in addition to fat, and it must modify the composition and density of the fat-free mass. The question is not whether this is the case but what are the nature and magnitude of the alterations.

SYNTHETIC DIETS FOR RABBITS, GUINEA PIGS AND RATS

An important axiom of biochemistry is that the metabolic processes of all living things are basically identical, that special processes are superimposed on fundamentally equivalent metabolic patterns. A corollary of special significance to the nutritionist is that the metabolic fuels and regulators are basically the same for all living things. In spite of the remarkable similarity of the nutritional requirements of different species, there is much to be gained from the use of a variety of experimental animals. Among the striking illustrations of this point one may cite the ascorbic acid requirements of the guinea pig, monkey and man. One of the principal requirements for the study of the nutritional requirements of any species is a highly purified ration which as a minimum condition will support satisfactory growth of the young and maintenance of the adult. Such additional criteria of nutritional adequacy as support of reproduction and lactation must often be ignored because of the difficulty of devising purified rations which will be adequate for these purposes.

J. G. Wooley (*J. Nutrition* **52,** *39 (1954)*) has reported on the use of purified diets for the rabbit. In an earlier study (Wooley and W. H. Sebrell, *Ibid.* **29,** *191 (1945)*) 6 to 8 week old rabbits were successfully placed on purified diets. Moderate growth rates were reported. In the present study it was shown that 3 to 4 week old rabbits could be used with partial success.

New Zealand white male rabbits were weaned at 3 to 4 weeks of age, housed in individual wire-bottom cages and fed one of the experimental rations. The first diet containing 20 per cent casein, 55.6 per cent sucrose, 15 per cent cellophane, 5 per cent fat, 4 per cent salts, plus vitamins supported only very slight growth. Supplementation with 40 per cent dried powdered kale greatly increased the growth performance. Another purified diet, similar to one formulated by M. E. Reid and G. M. Briggs (*J. Nutrition* **51,** *341 (1953)*), contained 30 per cent casein, 37 per cent carbohydrate (a mixture of sucrose, glucose and starch), 15 per cent cellophane, 7.3 per cent fat, 6 per cent salts with added potassium acetate and magnesium oxide, as well as vitamins. This diet, containing more casein, salts and fat and less carbohydrate than the first one, differed also in that it contained ascorbic acid, para-aminobenzoic acid, folacin, biotin and vitamin B_{12}. In addition it also contained larger amounts of some of the other vitamins. This ration, without further supplement, supported growth almost as well as the first diet when the latter was supplemented with kale. Supplementation of the more adequate diet with kale did not improve the growth of the experimental animals. Growth over a four-week period was, however, inferior to that observed with a pelleted stock ration. A variety of vegetable materials showed some growth stimulation when tested under the conditions in which kale was effective. Since such supplements would furnish, among other things, large amounts of potassium salts, the author tested potassium acetate and bicarbonate for growth stimulation. While these salt supplements were somewhat effective, they did not enhance growth to the same degree as an equivalent amount of kale. Substituting cornstarch for some of the sucrose of the first diet resulted in some growth stimulation. A 30 per cent casein level supported greater growth than a 20 per cent level providing additional potassium was supplied.

This work shows in a convincing manner that a synthetic diet for the rabbit should contain between 25 and 30 per cent protein (if casein is used), and that the diet should

contain more potassium than diets for the rat. It is clear that vegetable materials, particularly kale, stimulate growth, an effect due only partly to the added potassium supplied by such supplements. However, even kale does not furnish all the necessary conditions for maximum growth.

The pantothenate requirement of the guinea pig has been investigated with the aid of a successful synthetic diet by M. E. Reid and G. M. Briggs (*J. Nutrition* **52,** *507 (1954)*). Guinea pigs weaned one to twenty-one days after birth were fed a synthetic diet containing 30 per cent casein, 7.3 per cent fat, 6 per cent salts, several carbohydrates and liberal quantities of vitamins, except pantothenate. In the presence of ascorbic acid but in the absence of pantothenate growth was poor and mortality high. The fur became rough, and the animals tended to develop diarrhea, anorexia and weakness. The porphyrin stains on fur and whiskers, commonly seen in the rat, were absent. In animals with patches of black fur some dulling of the pigment was seen, but actual loss of pigmentation did not occur. Adrenal enlargement, hyperemia and hemorrhage were observed.

Guinea pigs placed on the deficient diet when 2 to 4 days old rapidly developed the symptoms of deficiency and died, while animals started on the ration when somewhat older survived longer and required more time for the development of deficiency symptoms. The minimal dietary level of pantothenate for maximal growth appeared to be 15 mg. per kilogram of diet, although the requirement depended somewhat on the age of the animals at the start of the experiment. Guinea pigs placed on the diets at an early age showed a somewhat larger requirement for the vitamin.

A study of the possible interrelationship between pantothenate and ascorbic acid showed that animals deficient in both factors died earlier than those lacking only one of them. On the other hand the addition of large amounts of ascorbic acid to the ration did not ameliorate the pantothenate deficiency.

A report on more than twenty years of experience with a stock ration for the maintenance of a rat colony was made by R. B. Hubbell (*J. Nutrition* **53,** *429 (1954)*). Several reports on the effect of diet on the growth performance of the Connecticut Agricultural Experiment Station colony have appeared in the literature over several decades. The Hubbell paper contributes importantly to this series. The basic stock ration at the Connecticut experiment station is G.L.F. Calf Meal. Supplements of dried yeast and wheat germ are used. Lactating females and newly weaned young receive a "paste food" consisting of 25 per cent casein, 25 per cent whole milk powder, 20 per cent wheat germ, 24.5 per cent lard, 5 per cent starch and 0.5 per cent cod liver oil. Under these conditions the weaning weights of the young are excellent, as are the reproductive and lactational performances of the breeding females. The use of a lactation supplement is strikingly effective, a finding in agreement with several reports from other laboratories.

Another group of animals is maintained on a special ration in preparation for the vitamin D line test. Weight losses of the lactating females and lower weaning weights of the young indicate that this diet is less satisfactory in supporting reproduction and lactation.

Weaning weights varied inversely with the number of young in the litter and depended upon the position in the litter series. The data in the paper were taken from observations of thousands of animals. While the data were obtained with stock rations, an optimal synthetic diet must be able to match the growth, reproduction and lactation support of such stock rations.

A FACTOR IN OIL MEALS AFFECTING ANIMAL FAT COMPOSITION

The unsaturated fatty acid composition of the dietary fats is the primary factor affecting the fatty acid composition of carcass fats in animals. When animals are fed vegetable oils or vegetable oil meals high in 2-double bond (diethenoic) or 3-double bond (triethenoic) unsaturated fatty acids, the carcass fat will contain increased amounts of these unsaturated fatty acids. Carcasses with increasing amounts of these unsaturated fatty acids become progressively softer in texture because of the lower melting point of the fat. This is of major importance, particularly in pork production, since pork of soft texture is generally not so acceptable as firm pork.

Not all the fats behave in the above manner. Cottonseed oil, for example, when fed in small amounts in the form of cottonseed meal to swine produces carcass fat that is firmer than that obtained on control rations. Increasing the cottonseed meal to a level above 15 per cent of the ration produces a distinct softening effect of carcass fat. These observations (E. H. Hostetler, J. O. Halverson, and F. W. Sherwood, *North Carolina Agr. Exp. Sta. Tech. Bull. No. 61 (1939)*) served as a basis for investigating the influence of dietary protein on the unsaturated fatty acid content of carcass fats in rats (S. B. Tove, F. H. Smith, C. T. Young, and Sherwood, *J. Nutrition* **54,** *49 (1954)*).

Weanling rats were fed the diets for six to seven weeks and were then analyzed for unsaturated fatty acids. The diets were of the purified type and contained 5 per cent of cottonseed oil and 23 per cent of protein.

When the dietary protein consisted of ether-extracted soybean meal or cottonseed meal, the carcass fat contained 23 to 24 per cent of monoethenoic acids, 28 per cent of diethenoic acids, 0.4 to 0.5 per cent of triethenoic acids and 2.5 to 2.8 per cent of tetraethenoic acids. Replacement of these proteins with egg albumin, casein, fibrin, or soybean alpha-protein plus methionine resulted in a distinct lowering of the diethenoic acids and an increase in the monoethenoic acids of the carcass fat. With these dietary proteins, for example, the monoethenoic acids represented 28 to 30 per cent of the total fatty acids in the carcass fat and the diethenoic acid level was 20 to 22 per cent. The percentages of triethenoic and tetraethenoic acids were also lowered with the egg albumin, fibrin and alpha-protein diets.

To rule out the possibility that residual traces of unsaturated fatty acids still present in ether-extracted soybean or cottonseed meal could be responsible for the difference in behavior of these proteins and the other protein sources used, ether extracts of acid hydrolysates of soybean meal were fed as supplements to the egg albumin diet. The feeding of this extracted residual soybean lipid had no influence on the unsaturated fatty acid content of carcass fats.

An attempt was made to extract the factor in the soybean meal that was responsible for the higher diethenoic acid content of carcass fats. Feeding 70 per cent methanol-extracted soybean meal resulted in essentially the same carcass unsaturated fatty acid composition as was observed with meal not treated with methanol. The addition of the methanol extract to the egg albumin diet, however, resulted in an increase in carcass diethenoic acid and a lowering of the monoethenoic acid content comparable to that obtained with ether-extracted soybean meal.

These results suggest that soybean and cottonseed meals, after ether extraction, contain factors that favor the deposition of diethenoic acids in rat carcass fat and that such factors are absent in more purified proteins such as egg albumin, casein, fibrin, or purified soybean protein. The nature of these factors remains to be determined.

NOTES

The Determination of Serum Albumin Concentration

Many chemical and physical methods are available for determination of the plasma proteins. Those usually available in hospital laboratories depend upon separation of albumin and globulin by salting-out procedures. The inaccuracy of these methods is well known when abnormal relationships between the albumin and globulin concentrations exist. The importance of albumin as an indication of nutritional status and of liver function cannot be underestimated. Hence, it is pleasant to find a relatively simple yet accurate method for the determination of the albumin concentration in serum and plasma.

The method was developed by D. D. Rutstein, E. F. Ingenito, W. E. Reynolds, and J. M. Burke (*J. Clin. Invest.* **33,** *211* (*1954*)), and depends upon the selective binding of an azo dye to human albumin, with measurement in a colorimeter or spectrophotometer of the color change of the dye after binding. The authors show figures substantiating a linear relationship between albumin concentration and optical density over a satisfactory range. They found further that other plasma fractions did not react with the dye nor interfere with the albumin-dye combination. The method was found to be reproducible and gave a mean albumin concentration of 4.1 g. per 100 cc. in 30 normal subjects. Generally, good agreement was found by this method and electrophoresis.

Clear serum was essential and in a number of instances lipemic serum was centrifuged at high speed for clearing before determination.

The results indicate that this method should be highly useful clinically. More work is required to establish its validity when wide variations from normal serum proteins occur, and in various diseases and after therapeutic agents which might interfere with the dye-albumin combination.

Use of Radiation in Food Processing

Foods are processed for two major reasons: to inactivate enzymes and to kill harmful microorganisms. Any satisfactory agent must also produce no objectionable flavors and should not produce chemical alterations which would affect the nutritive value of the food. The most widely used processing agent is refrigeration. There are several objections to this method. The material must be kept in a frozen state and this becomes a problem when food is shipped long distances as for example in supplying troops. In recent years there has been considerable interest in the use of radiation in food processing. Radiation will inactivate enzymes and will kill microorganisms, although enormous doses are required. The possibilities and problems of radiation processing of food are discussed by B. S. Schweigert (*J. Am. Dietet. Assn.* **30,** *973* (*1954*)).

It is pointed out that many problems must be solved before this method can have widespread application. One of the problems is the production of undesirable flavors as a result of radiation treatment of foods, particularly meats. However, research in progress may solve this problem. One promising approach is the addition of ascorbic acid to the food during the radiation process. The most noteworthy consideration is the magnitude of the research effort directed toward the use of radiation for food processing. It seems quite probable that in the future radiation processing of food will have widespread application.

Nutrition and Highway Safety

"Human Factors in Highway Transport Safety" is the subject and title of a recent publication from the Harvard School of Public Health (*Boston* (*1954*)) prepared by Ross A. McFarland and Alfred L. Moseley of the Department of Industrial Hygiene and dealing primarily with truck and bus operation.

Of particular interest to nutrition workers

are sections on the effects of alcohol on behavior and on the physiologic functioning of the body, and the relation of diet to driving efficiency. A survey of the food habits and diets of local and long distance truck drivers indicates a consistent tendency toward low intakes of calcium, vitamin A, riboflavin, ascorbic acid and roughages, along with a tendency to eat large starchy meals, the latter contributing to the dangers of after-meal sleepiness. Work schedule governs to a large extent the content of the meal and the time at which it is eaten. Unusual working hours and skipped meals have their effects on digestion and nutritional status. Recommendations for better diet habits and meal patterns to fit the needs of truck and bus drivers and to improve their efficiency are outlined.

Another study of this series published by the Department of Industrial Hygiene of the Harvard School of Public Health, "Human Body Size and Capabilities in the Design and Operation of Vehicular Equipment," gives anthropometric data which may be of interest to nutritionists. Based upon anthropometric studies of representative groups of 360 commercial drivers and 2500 military drivers, the former exhibited a significant increase over military drivers in such body dimensions as abdominal depth and seat breadth. Body circumferences in general, and weight, were higher for the commercial drivers.

Fetal and Maternal Riboflavin

J. E. Lust, D. D. Hagerman, and C. A. Villee (*J. Clin. Invest.* **33,** *38 (1954)*) have analyzed maternal and cord blood at term for free riboflavin and riboflavin mononucleotide, flavin adenine dinucleotide and total riboflavin. They found a four-fold increase in free riboflavin in the fetal blood, some reduction in the flavin adenine dinucleotide, and little of the mononucleotide in either of the specimens, producing a distinct although not marked increase in total riboflavin content of the fetal blood. Analysis of the placenta revealed considerable amounts of riboflavin in the placenta, most of which was the dinucleotide. Minced placenta was shown to be capable of splitting the dinucleotide. Finally, a twenty-week fetal liver had a high concentration of riboflavin in the form of the dinucleotide.

These data taken together led the authors to suppose that fetal tissues synthesize dinucleotide from free riboflavin transferred by the placenta against a gradient from maternal to fetal blood. Presumably the dinucleotide in the maternal blood was split by the placenta before its passage into the fetal circulation. This accumulation of riboflavin in the fetus, although not great, is reminiscent of the same phenomena for other nutrients, for example, amino acids (*Nutrition Reviews* **7,** *106 (1949)*).

Recent Nutrition Books

Food Composition Tables—Minerals and Vitamins. Charlotte Chatfield. Food and Nutrition Division, Food and Agriculture Organization. Price: $1.00. Copies may be Obtained from International Documents Service, Columbia University Press, 2960 Broadway, New York 27, N. Y.

The Problem of Providing Optimum Fluoride Intake for Prevention of Dental Caries. Food and Nutrition Board, National Research Council, Washington, D. C. Pp. 15. Price: $0.50. Order from Division of Biology and Agriculture, National Research Council, 2101 Constitution Ave., N. W., Washington, D. C.

Identification of Some Internal Parasites of Laboratory Animals. Robert T. Habermann, Fletcher P. Williams, Jr., and William T. S. Thorp. Public Health Service Publication No. 343, Washington, D. C., 1954. Pp. 30. Price: $0.20. For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.

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